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Use of Peptoid Microspheres to improve Enzyme Linked Immunosorbent Assay (ELISA) Microarray for Early Cancer Detection

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Use of Peptoid Microspheres to improve Enzyme Linked Immunosorbent Assay (ELISA) Microarray for Early Cancer Detection

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Abstract

The pursuit of an efficient and economical technique for early detection of cancer has led to the development of sophisticated biosensors for antigen analysis. As biosensors become more complex, coatings are necessary and important. The preferred coatings need to have the following properties: high surface area for binding, resistance to biofouling, and flexible synthesis. The focus of this project is to determine whether using peptoid microsphere-coated glass slides in the enzyme linked immunosorbent assay (ELISA) microarray technique is better than using commercially available glass slides. Current microarray slides are coated with reactive groups, such as amines, epoxides, or aldehydes. It is believed that using three-dimensional coatings will allow for increased binding efficiency of the capture reagent, and therefore increased dynamic range and sensitivity. Helical peptoids that are partially soluble in water have been shown to form microspheres when dried on a solid surface. In this project, peptoids will be synthetized, purified and characterized. Each peptoid will be dissolved in a protic solvent to stabilize the secondary helical structure and promote microsphere formation. The peptoids solutions will be administered onto the ELISA microarrays glass slides and allowed to dry in order to form uniform microsphere coatings. The peptoid microsphere-coated glass slides will be tested and compared against commercially available glass slides for use in ELISA microarray.

Introduction and Background

The pursuit of an efficient and economical technique for early detection of cancer has led to the development of sophisticated biosensors for antigen analysis.[2] Biosensors are becoming more complex, and for this reason, coatings are necessary and important because creating antibody biosensors of great diversity is very expensive.[2] The preferred and desired coatings need to have the following properties: high surface area for binding, resistance to biofouling, and flexible synthesis.[4] We believe that this coating can be achieved using peptoid microspheres.

The ultimate goal of this research is to compare enzyme linked immunosorbent assay (ELISA) microarray using peptoid microsphere-coated or commercially available glass slides in order to determine which is more efficient for cancer detection. A microarray is a tool used to sort through the information contained in a genome or proteome. ELISA microarray uses a capture antibody to specifically bind to and immobilize analyte from solution. Current microarray slides are thinly coated with reactive groups including amines, epoxides, or aldehydes. We hypothesize that three-dimensional coatings will allow for increased binding efficiency of the capture reagent. This will lead to an increased dynamic range and potentially increased sensitivity. Sandwich ELISA uses capture antibodies to bind to antigen, and then detection antibodies to bind to another epitope. Detection antibodies have a fluorescent tag, and binding to analyte shows fluorescence under wavelength-specific excitation after the signal has been amplified.[3]

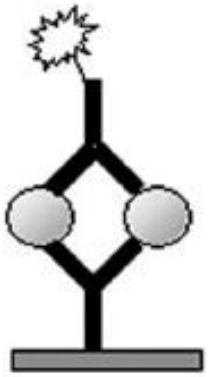

Figure 1. Sandwich ELISA.[3]

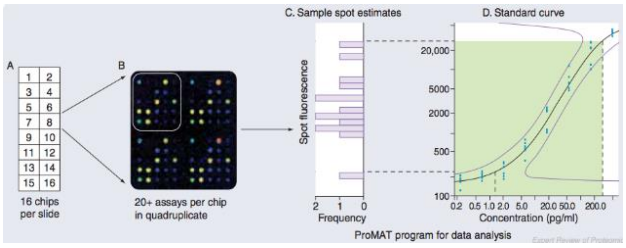

Figure 2. Prototype for ELISA microarray analysis. [2]

Sandwich ELISA microarrays method of application in our lab works as shown in figure 2. Fig. 2A shows the total of 16 identical chips that are printed on a single glass slide. Each chip can contain 20+ assays printed in quadruplicate. Fig. 2B shows a fluorescence image of a chip after processing, with high intensity fluorescence indicated from blue to red. Once the fluorescence intensities have been quantified, the data is analyzed using ProMAT to create a plot of relative fluorescence vs. antigen concentration (Figs. 2C and 2D).[1] Fluorescence is directly correlated with antigen concentration. This means, the higher the intensity of the fluorescence, the higher the concentration of the antigen. The problem with ELISA microarray is that the range it provides can be limited. This dynamic range can be improved by increasing the surface area, and therefore binding more capture reagent to the slide (indicated by the dashed line in Fig. 3).

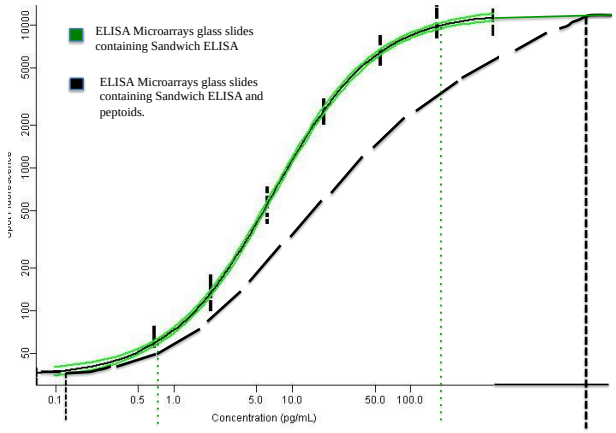

Figure 3. Projected visual comparison between ELISA Microarrays glass slides and glass slides with microspheres

In order to be practical in biosensor applications, coatings must be able to withstand multiple conditions. In this project, the microspheres were formed and stored in different conditions. Then, the microspheres were monitored to determine how the conditions affected the microspheres. The focus of this project was the ability of the microspheres to withstand the

storage conditions instead of the uniform formation of the self-assembling peptoid microspheres coating. Based on the ultimate goal of the project, this focus is important because it is necessary to find an ideal condition of storage for the peptoids microspheres.

Carefully designed peptoids, or poly-N-substituted glycines, have been shown to self-assemble into microspheres, which can be used to increase the surface area of ELISA microarray slides.[2] Peptoids are biomimetic polymers with a backbone similar to that of peptides (Fig. 4).[1] Unlike peptides, peptoid side chains are attached to the amide groups rather than the α-carbon of their backbone.[1]

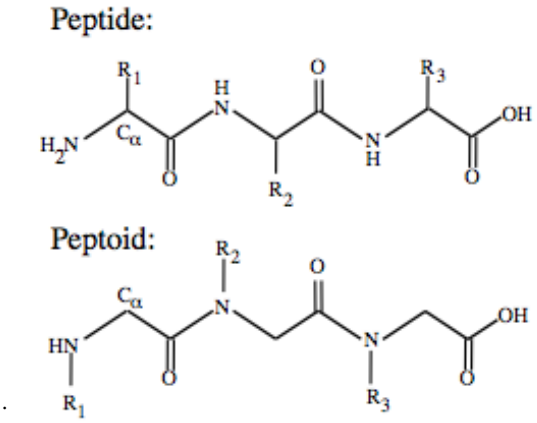

Figure 4. Peptide and Peptoid Structures. [6]

Other molecular features of the peptoids are achiral backbone and no H-bond donors in the backbone. Peptoids have two main advantages over peptides. These advantages are protease resistance and low immunogenicity. Peptoids can be used as coatings on slides for ELISA microarray because of their ability to increase surface area through supramolecular assemblies, their ease of synthesis, and the numerous available side chain chemistries.[4] Increasing the surface area of the glass slide will allow detection of small quantities of antigens in a specific sample.

Methods and Materials

Peptoids that have helical secondary structure and are partially soluble in water have been shown to self-assemble into microspheres.[2] This ability of self-assembling can be controlled by changing the peptoid chemistry.[4] Altering the side chain chemistry can led to different size microspheres.[2] The peptoid used in this project was designed with interchanging positively charged and aromatic groups on the third face of the helix. Figure 5 shows the structure of the peptoid used in this project with its molecular weight on the bottom right corner. The inclusion of chiral aromatic side chains induces the helical structure, increases the bulkiness of the peptoid and maintains water solubility. Figure 6 shows a schematic representation of the peptoid microspheres.

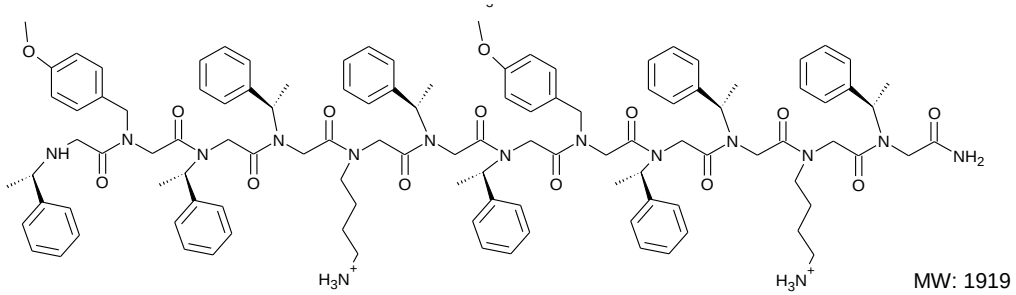

Figure 5. Designed Peptoid for this Research project. [4]

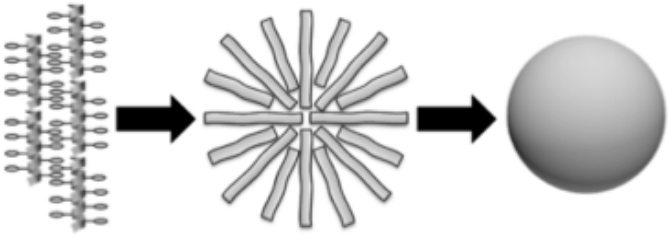

Figure 6. Schematic representation of Peptoid microspheres. [2]

The peptoid was manually synthesized and the first step was to react rink amide resin with bromoacetic acid, which created the backbone of the peptoid. An S_N2 substitution reaction was used to add an amine group as the functional side chain. This cycle was repeated as many times as needed until the desired peptoid sequence and chemistry is reached (See Fig. 7).[1] In this case, it was repeated twelve times. Peptoid was cleaved from the rink amide resin by bathing in a solution of 95% triflouroacetic acid, 2.5% triisopropylsilane and 2.5% water for two to ten minutes. Then, the acid was removed via a Heidolph Laborota 2001 rotating evaporator, shown in Figure 8.

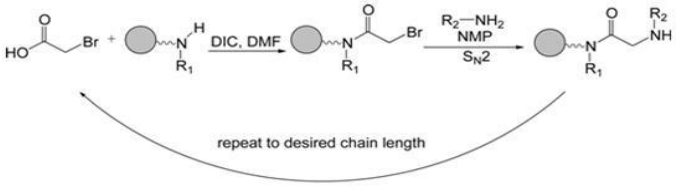

Figure 7. Peptoid Synthesis.[6]

Figure 8. Heidolph Laborota 2001 rotating evaporator.

The peptoid was diluted to a concentration 3 mg/mL in a 50:50 solution of acetonitrile-water. Then, this sample was injected and purified by preparative reversed phase high pressure liquid chromatography (HPLC) using a Waters Delta 600 system with a Duragel C18 column and a gradient of water and acetonitrile (35%-95%) for 60 minutes. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI) was used to confirm the molecular weight of the fractions collected in order to determine the fractions that belonged to the peptoid.

Purity greater than 97% was confirmed by analytical reversed-phase HPLC using a Waters Alliance system with a Duragel C18 column and a gradient of water and acetonitrile. The analytical reversed-phased HPLC also allowed determining pure fractions of the peptoid to combine. Final mass was confirmed by MALDI.

The peptoid was dissolved in a 4:1 solution of ethanol and water to a 5 mg/mL concentration. [4] Protic solvents help to stabilize the secondary helical structure of the peptoids through hydrogen bonding.[5] These peptoids solutions were administered onto wax imprinted glass slides using the full coverage technique (20 μL), previously shown to have the most influence on the uniformity of the microsphere coating.[4] In this technique, large samples of volume are applied to the glass slides using a pipette and fully coating the surface.[4] Peptoid dissolved in solution was left to dry at room temperature with relative humidity of 60%, which helped to form uniform microspheres.[4] The presence of peptoid microspheres was confirmed using scanning electron microscopy (SEM). The peptoid microspheres coated glass slides were then storage at different conditions (- 4 °C, -20 °C, room temperature and presence of desiccant) and the robustness was monitored over a specific time span using SEM. SEM shoots electrons at gold nano particle coated samples to produce a 3D image of the surface. The samples were coated with gold nano particles in order to have a higher conductivity.

Results and Discussion

The peptoid was purified using a preparative high performance liquid chromatography (HPLC). The peptoid peak was then identified (red rectangle in Figure 9.)

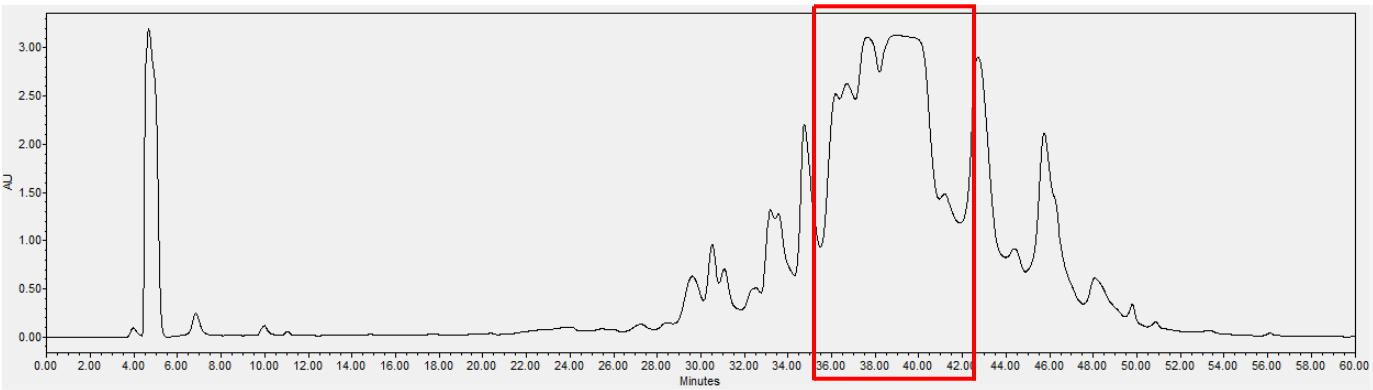

Figure 9. High Performance Liquid Chromatography (HPLC) results

MALDI was used to confirm final molecular weight, 1919. This result, Figure 10, matched the expected molecular weight.

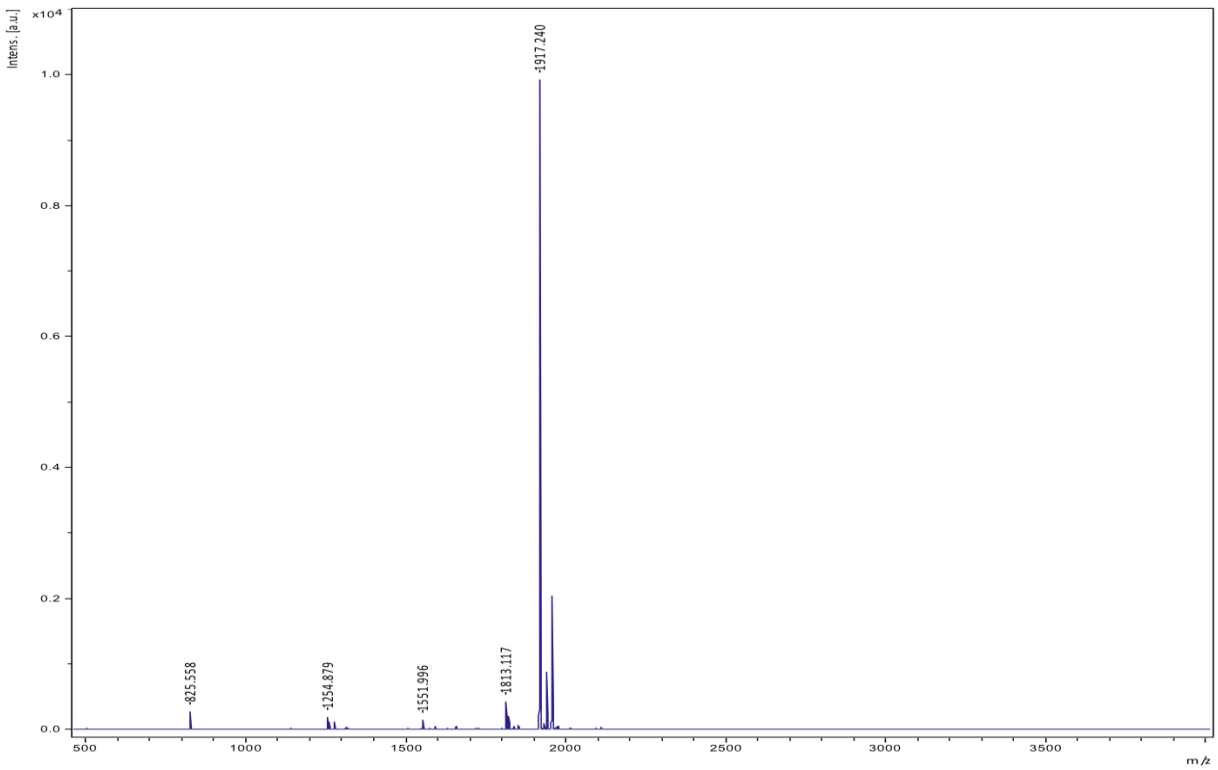

Figure 10. MALDI results

Purities greater than 97% were confirmed by analytical reversed-phase HPLC. The purities greater than 97% can be found inside the red rectangle in Figure 11.

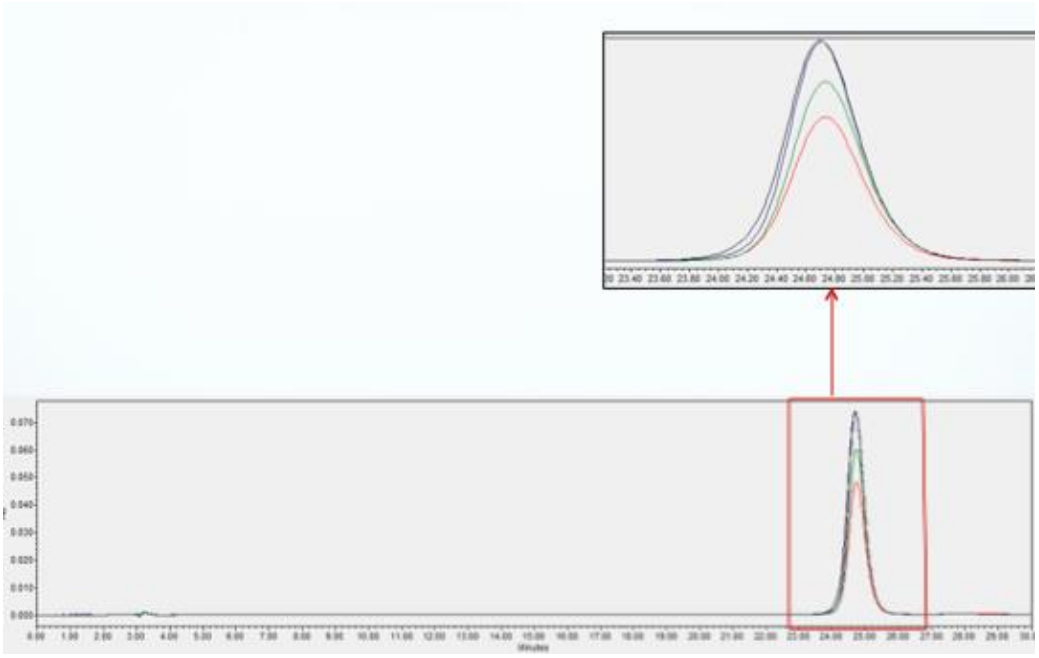

Figure 11. Analytical Reversed-phase HPLC results

Day One

Four different sets of glass slides were coated with the peptoid solution. The solution was allowed to dry at room temperature and 60% relative humidity. The presence of peptoid microspheres on the glass slide was confirmed using scanning electron microscopy (SEM).

According to Figure 12, peptoid microspheres were formed on the glass slides. Then, the microspheres coated glass slides were stored in the four different conditions (-4 °C, -20°C, room temperature, presence of desiccant)

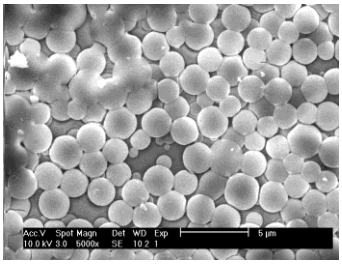

Figure 12. Result from day one observed at 5 µm.

Day Four

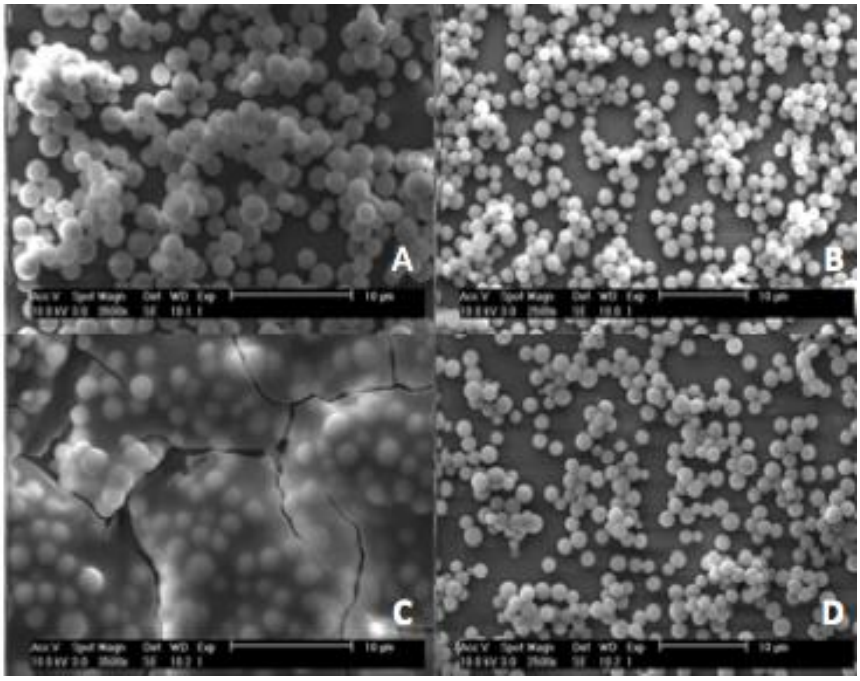

Figure 13. Results from day three observed at 10 µm (A. -4 °C, B. -20°C, C. room temperature, D. presence of desiccant)

Day Seven

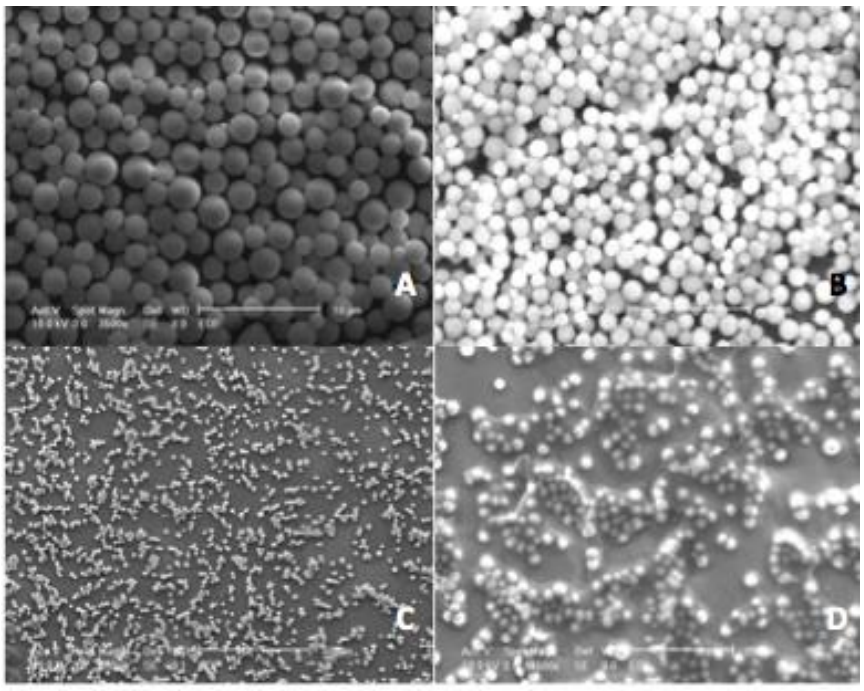

Figure 14. Results from day seven observed at 10 μm (A. -4 °C, B. -20°C, C. room temperature, D. presence of desiccant)

SEM was used to monitor the microspheres on the glass slides. Peptoid microspheres were monitored after being stored for four and seven days. The presence of microspheres was not affected by the different conditions but the overall coating was. This project focused on how the microspheres were affected by the conditions and according to Figure 13 and 14, the microspheres were able to withstand the four different conditions. Based on Figure 13.C and 14.C, a layer was formed on top of the microspheres. Therefore, room temperature would be a less ideal condition to store the microspheres coated glass slides. According to these results, the ideal conditions could be either at below freezing temperature or room temperature with the presence of a desiccant. Further studies are neceesary in order to determine an optimal storage condition for uniform peptoid microspheres coatings.

Conclusions

Peptoids, or poly-N-substituted glycines, can be designed for a multitude of applications due to easy synthesis. Peptoids that have helical secondary structure and are partially soluble in water can self-assemble into microspheres. Peptoids microspheres can be used to increase the surface area of ELISA microarray slides. Increasing the surface area of the glass slide will allow detection of small quantities of antigens in a specific sample. The peptoid was designed, synthesized, purified, characterized, and dissolved in a 4:1 protic solvent and water solution. Ethanol was a good protic solvent for the formation of uniform self-assembling microspheres. Peptoids microspheres were formed on comercially available glass slides and stored in different conditions. Even though the microspheres coating was affected by the four different storage conditions, the self-assembling microspheres were able to withstand the storage conditions. Room temperature storage was less ideal since a layer was formed on top of the microspheres coating.

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